CHEMOSENSORY BASES OF FOOD-FINDING AND FEEDING IN APLYSIA JULIANA (MOLLUSCA, OPISTHOBRANCHIA)[1]

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While many opisthobranchs have special feeding preferences, the sensory mechanisms involved in their food choices are mostly unstudied (Kohn, 1961; Paine, 1963). *Aplysia juliana,* a common sea-hare of Hawaiian waters, is almost monophagous, feeding only on *Ulva lactuca,* if available, but taking *Ulva fasciata,* if given only this. On the latter, however, the animals do not grow normally. These sea-hares may nibble on other algae, but never eat enough to grow or survive for long. They live well in marine aquaria when fed *Ulva lactuca,* and make good subjects for research on sensory physiology. The animals we tested were maintained in the laboratory in ten-gallon aquaria or one-gallon jars furnished with sub-sand filters. Sea water was obtained locally and used unfiltered. If given food regularly, the animals usually lived for 2–4 months, and grew from a few millimeters to 12–18 cm. long. This report describes the responses of *Aplysia juliana* (hereafter, aplysia) to its food plant, *Ulva lactuca* (hereafter, *Ulva*), with data on sensory processes involved.

RESPONSES TO ULVA

If aplysias are without food for a few days, they usually bury in the sand and remain hidden. One may, on looking into an aquarium, be quite unaware that any of these animals are present. If he drops a small piece of *Ulva* into the water, within 10–15 seconds the oral tentacles of the animals appear, followed by the heads, as the sand seems to come alive, and the aplysias crawl out, with tentacles spread (Fig. 1). They climb the sides of the aquarium, holding fast with the posterior sucker that is characteristic of this species, and extend their tentacles as if sniffing the water, as a dog sniffs the air. If an aerator is in action, the animals may seem not to orient well, but if the water is relatively quiet or moving in a specific direction, they go fairly directly toward the *Ulva.* Upon touching it, they immediately seize it with the mouth and commence feeding.

The response to food occurs, therefore, in three major steps—arousal, orientation, and feeding, as in many predatory and carrion-feeding gastropods (Kohn, 1961). One is impressed, on first seeing the arousal and orientation, by the rapidity and precision of motion for these animals, and the obvious use of the oral tentacles. On contact with the *Ulva,* the aplysias react even more rapidly, as if they were using a different sensory modality. Certain questions immediately come to mind: (1) What is given off by the *Ulva* that attracts the animals? (2)

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With what organ or organs is the stimulus received? (3) How is this behavior related to the general feeding behavior of the animals?

Nature of the Attractive Substance

It was fairly obvious that only the chemical senses were involved in these reactions. Dropping pieces of other algae or other objects into the water did not arouse quiet animals, thus eliminating mechanical effects. The eyes of these animals would hardly seem to be involved, but to eliminate this possibility the following test was made. A few blades of *Ulva* were put in fresh sea water for about ten minutes, and only the water was then added dropwise to an aquarium holding the aplysias. This proved just as stimulating as the *Ulva* itself. As few

FIGURE 1. Young *Aplysia juliana* (12 mm. long) in presence of material from *Ulva lactuca*. Note spread oral tentacles, posterior sucker of foot, and characteristic seeking posture.

as two drops (about 0.2 ml.) of this water added to about 30 liters of sea water in the aquarium—a dilution of one part to 150,000 in terms of water alone; at least 1 in 15,000,000 (assuming the almost certainly high value of 1% dissolved material) in terms of the material—brought the animals forth within 10–15 seconds. Drops of plain sea water, before *Ulva* was added, had no effect when added to the aquaria.

All further experiments were performed with this *Ulva*-water—fresh sea water in which blades of *Ulva* had stood for five or more minutes—for this eliminated other than the chemical senses. Freshly gathered *Ulva* was best for this. If the *Ulva* stood in the laboratory, particularly if in a crowded container, it lost its attractiveness within a few days. Rotting, no matter how little, reduced or abolished the attractiveness.

Preliminary attempts were made to determine the chemical nature of the attractive substance. First, its possible volatility was tested. Air was bubbled through a bottle containing sea water and *Ulva*, and then led through tubing to a bottle containing hungry aplysias. No matter how long or under what circumstances this was done—including varying speeds of air flow, introduction of an air-breaker to break up the air current into bubbles, using small or large quantities of *Ulva*, warming the sea water containing the *Ulva*—the aplysias did not react. In every case, placing a few drops of the sea water covering the *Ulva* into the

FIGURE 2. Response of *Aplysia* at surface of water to *Ulva*-water dropped from pipette at right onto mouth.

bottle with the aplysias aroused them almost immediately. *Ulva*-water was boiled for 15 minutes, and then tested. It was as effective as the original solution, as determined by comparative dilution tests—diluting the *Ulva*-water before and after boiling with successive additions of sea water and retesting. Dilutions of five to seven times were possible with both solutions before the effectiveness was lost.

Ether extraction was tried, using a separatory funnel and shaking with ether for 15 minutes. The material remained in the water fraction, and that could be boiled, after the extraction, for 15 minutes and still be as potent as before. The tests so far conducted do not allow much to be said about the chemical nature of the material, except that it seems more like substances associated with contact chemoreception—*i.e.,* non-volatile, water-soluble, heat-stable—where separation of contact and distance chemoreception is possible.

Receptive Areas on the Body

To discover the receptive loci for the chemical, *Ulva*-water was allowed to flow gently from a finely drawn pipette onto specific areas of the body of aplysias which had been starved for four or five days. Control tests, using ordinary sea water from a duplicate pipette, were conducted before each experimental test. The following regions of the body were found to be non-receptive: anywhere in the mantle cavity, on the foot, or on the surface of the parapodia, near the anus, the rhinophores, and between the rhinophores and near the eyes. The only areas whose stimulation resulted in orientation and feeding responses were the oral tentacles and the mouth.

The reaction to stimulation of the tentacles is an excellent index of reception. An animal immediately extends the tentacles toward the pipette, then raises its head and reaches for the pipette with its mouth. If the animal is near the water surface, with the foot along the surface. *Ulva*-water can be dropped onto the mouth, and this elicits radular action, as in feeding (Fig. 2). An animal can thus be led around at the surface, with the radula sweeping out regularly. The material, therefore, besides acting as an orienting stimulus, apparently through receptors on the tentacles, also acts as a phagostimulant when applied to the mouth.

The phagostimulant action of *Ulva*-water is surprisingly intense. The aplysias eat filter paper soaked with *Ulva*-water, even though this is much coarser than their ordinary food. Furthermore, they eat other algae, if given *Ulva*-water while the algae are applied to the mouth. Some even try to eat sea anemones that are in the way at the surface, when stimulated by drops of *Ulva*-water on the mouth. If hungry aplysias are mating or laying eggs, drops of *Ulva*-water nearby cause them to stop and to seek the source of the stimulation.

The lack of sensitivity of the rhinophores is worthy of comment, for these organs have been thought by many biologists to be olfactory in function. The name itself implies this, and was so meant (Bergh, 1864). Early comparative anatomists believed that the innervation and cellular structure are like those of the olfactory organs of vertebrates. An olfactory function for the rhinophores is still stated in some general literature, in spite of the reports of Arey (1918), Crozier and Arey (1919), and Agersborg (1922) to the contrary. Stimulation of the rhinophores of aplysias with a current of water brings about turning of the head toward the current if it is gentle, or away if it is strong. The tentacle on the side stimulated is raised and directed toward the current. This reaction is slow enough that it is easy to follow. If the current is of plain sea water, the tentacle ripples through it, then the animal returns to its ordinary behavior. If the current contains the *Ulva*-factor, the animal follows the current as soon as the tentacle intercepts it—but not before. Thus, the rhinophores are sensitive to currents, as Arey and Crozier reported, and the tentacles are chemically sensitive, at least as far as this phagostimulant is concerned. It should be noted that Crozier and Arey, and Agersborg found the rhinophores sensitive to inorganic salts, acids, etc., not to volatile organic compounds, but so were almost all parts of the body. As Kohn (1961) has noted, the relevance of these studies to normal behavior may be questionable.

Discussion of Results

It is obvious that a water-soluble material given off by *Ulva* into sea water acts as a powerful attractant, enabling the aplysias to find food. Either the same or another water-soluble material acts as a strong phagostimulant, inducing feeding even on unnatural foods. These act at remarkably low concentrations, dilutions of at least 1 to 15,000,000. The chemical nature of the material or these materials remains to be determined. No attempt can be made, therefore, to designate it or them as contact or distance stimulants (Kohn, 1961).

The receptors for the material acting in arousal and orientation are on the tentacles, and may be confined to these, although receptors in the mouth could be active. The rhinophores are not sensitive to this material, strengthening the conclusions of Arey (1918), Crozier and Arey (1919), and Agersborg (1922) that the name, rhinophore, must not be thought to denote an olfactory function. All critical experimental evidence shows that the rhinophores are primarily receptors for mechanical stimuli, with no more sensitivity to inorganic materials than other parts of the body surface.

Stimulation of the oral chemoreceptors causes feeding behavior. The change in reaction from that elicited by stimulation of the tentacles suggests that a different stimulating chemical or some change in sensory modality may occur. No direct evidence for this was found, however, and it may be that the same material both attracts the animal and stimulates the oral receptors to elicit feeding. The exact receptors were not discovered, but they are near or in the mouth, for stimulation of the mouth alone, without stimulation of the tentacles, elicits feeding reactions.

The induction of feeding on filter paper and other unusual materials by stimulation of the animals with *Ulva*-water suggests that feeding is determined mainly by chemical stimuli. In a few cases, however, some of the animals refuse to feed on filter paper or other cellulose materials, even when flooded with *Ulva*-water, suggesting that mechanical factors also determine food selection.

Observations on Normal Feeding

In captivity, the aplysias generally consume all of the *Ulva* fed to them. Yet, in nature, no matter how many of the animals browse upon a bed of *Ulva,* they do not exterminate it. This observation led us to make some studies on feeding by these animals that indicate why this is so.

Ordinarily, when feeding captive aplysias, we tear *Ulva* from the rocks on which it grows to bring it to the laboratory. If, instead, we bring in rocks with the *Ulva* attached, the aplysias do not completely devour the *Ulva.* Instead they eat the succulent-appearing outer portions of the blades and leave the heavier bases. It is the former that are usually collected when the *Ulva* is merely pulled from the rocks. The basal parts are noticeably coarser (Fig. 3). On examining *Ulva* in regions with high populations of aplysias, we found that the plants that are large enough for feeding obviously have two parts—a basal, darker green, thicker, coarser section, and a distal, succulent, leaf-like portion. Ordinarily, the aplysias eat only the succulent growth; the basal, heavier portions are left. The bases produce new succulent blades within a week or two.

In the aquarium, the same thing is true. The aplysias eat the plants down to the heavy bases, but they do not eat these unless driven to it by long-continued starvation. In many cases, even this does not cause them to eat the bases. The aplysias can be induced to eat the bases by flooding their mouths with *Ulva*-water as they contact the bases. Apparently, if the phagostimulant is present in the basal parts, it is not given off, or is present in too low a concentration to excite the animals.

The ecological significance of this is obvious. By leaving the bases to regenerate new blades, the aplysias do not destroy their food supply completely, even when they become very numerous. They have considerable resistance to starvation, adults

FIGURE 3. *Ulva lactuca,* showing characteristic growth pattern (right), and coarse, basal portion (left) not eaten by aplysias, even though given no other food for several days; the blade was eaten within hours of being given to the animals.

living at least two weeks without any food. From an evolutionary standpoint, the situation is quite easily understandable, for the lack of phagostimulant in—or the coarser texture of—the bases saves these for replacement of the blades. The specificity of the aplysias to material from the terminal parts of the blades allows them ordinarily to find plenty of food. The ability of the animals to endure starvation with little weight loss enables them to survive easily during periods when rough seas or other accidents have torn off the edible portions of the *Ulva*. The bases produce new blades rapidly enough to save the animals from death by starvation. The presence or absence of a chemical substance attractive to an animal in different parts of the same plant may thus be responsible for maintaining the food supply of the animal against feeding pressure that would otherwise eliminate the food plant.

Summary

Sea-hares, *Aplysia juliana*, when hungry, are aroused to activity by and oriented toward *Ulva lactuca*, their normal food. The same response occurs when the animals are stimulated by sea water in which *Ulva* has stood for a few minutes. The material given off by the *Ulva* is non-volatile, heat-stable, and remains in the water fraction after ether extraction. The receptors for this substance, when it acts in arousal and orientation, are on the tentacles, but not on the rhinophores. When sea water in which *Ulva* has stood is dropped onto the mouth of an aplysia, the animal starts feeding activity. The stimulating material acts at very low concentrations and is strongly excitatory. Normally these aplysias eat only the terminal thin parts of *Ulva* and leave the coarser bases which produce new blades. This may be due to the absence of phagostimulant in the bases. The animals therefore do not eliminate the *Ulva*, even when they are very numerous, and their food supply is thus maintained.

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